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UEBER DIE OBERFLÄCHENAKTIVITÄT UND DIE ADSORBIERBARKEIT VON AMINOSÄUREN.

II. MITTEILUNG.

von

TAKEO ITO.

(Aus dem Agrikulturchemischen Laboratorium in Landwirtschaftlicher Hochschule, Morioka, Japan.)

(Eingegangen am 12 September 1930.)

In der vorhergehenden Mitteilung (Ito diese Zeitschr., **6**, 13～16, 1930.) wurde u.a. gezeigt, dass eine homologe Reihe von Aminosäuren——von Glykokoll bis Leucin——im grossen und ganzen entsprechend der Traubeschen Regel oberflächenaktiv und adsorbierbar sind. Von den genannten Aminosäuren sind, indessen, Valin und Leucin Isoverbindungen, während die andere normale Verbindungen sind. Es schien mir daher wunschenwert, die erwähnte Ergebnisse durch weitere Versuche mit den entsprechenden normalen Verbindungen zu ergänzen. Aus diesem Grunde wurde in der vorliegenden Untersuchung bearbeitet mit Norleucin (α–Amino–n–Capronsäure) und α–Amino–n–Valeriansäure. Ferner wurde Versuche an Prolin, welches ähnlich wie Valin bzw. dessen Isomer zusammengesetzt ist, vorgenommen.

Die zur Oberflächenspannungsmessung sowie für die Adsorptionsversuche angewandten Methoden waren ganz ähnlich wie bei der vorigen Arbeit (Lot. cit.). Nur bei Prolin hatte man zur Konzentrationsbestimmung seiner Lösung nach Kjeldahl zu arbeiten, da man hier bekanntlich nicht die Van Slykesche Methode anwenden kann.

Die Resultate sind in den folgenden zwei Figuren graphisch zusammengestellt.

In Fig. 1 sind die σ–C–Kurven von den drei angeführten Aminosäuren wiedergegeben, gleichzeitig sind die des Valins und des Leucins mit eingezeichnet. Als Ordinate ist die relative Oberflächenspannung σ ($\sigma_{H_2O}=1$) zugeordnet, als Abzisse die Konzentration C in Mol im Liter. Es ist ersichtlich, dass Norleucin und α–Amino–n–Valeriansäure merklich oberflächenaktiver sind als die entsprechenden Isoverbindungen.

Bei der ähnlichen Zusammensetzung ist Prolin (α–Pyrrolidincarbonsäure) auffallend weniger oberflächenaktiv als Valin bzw. dessen Isomer, was offenbar auf die Konstitutionsverschiedenheit zurückzuführen ist.

In Fig. 2 finden sich die Adsorptionsisothermen (Adsorbens : dieselbe Knochenkohle wie bei der schon mehrfach erwähnten Arbeit.). Als Ordinaten sind die adsorbierten Aminosäuremengen in Millimol pro g Kohle aufgetragen, als Abzissen die Gleichgewichtskonzentrationen C in Mol im Liter.

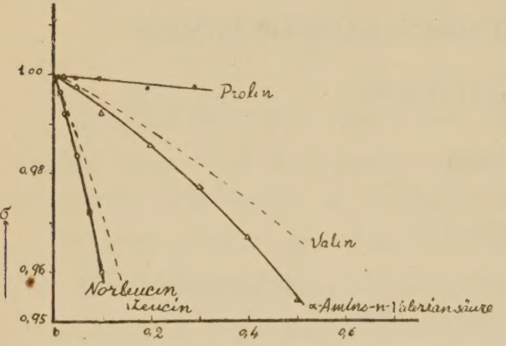

Fig. 1.
σ-c-Kurven von wäßrigen Aminosäurelösungen.

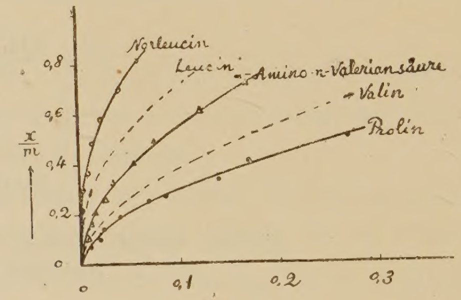

Fig. 2.
Adsorptionsisothermen von wäßrigen Aminosäurelösungen

Vergleicht man Fig. 2 mit Fig. 1, so erkennt man wieder einen deutlichen Parallelismus zwischen der Oberflächenaktivität und der Adsorptionsfähigkeit von den hier in Betracht kommenden Aminosäuren.

CHEMISCHE UNTERSUCHUNGEN ÜBER DAS GLUKOMANNAN AUS "KONJAK".

I. MITTEILUNG. DARSTELLUNG DES GLUKOMANNANS.

von

Kitsuji Nishida und Hideo Hashima.

(Eingegangen am 3 October 1930)

Nach den verschiedenen Mitteilungen liefert also das Konjak-Mannan nach seinen Untersuchungen bei der Hydrolyse drei Zuckerarten, Glukose, Mannose und Fruktose in verschiedenen Verhaltnissen, nämlich von 1:2:0, 2:5:0 und 2:3:1. Diese Ergebnisse wurden zur Ursache der Annahme für eine Uneinheitlichkeit in der Zusammensetzung des Mannan in den Händen gehabt.

Mannan kann durch kochendes Wasser oder durch Enzyme verflüssigt und durch Alkohol als Aufschlämmung ausgefällt werden, oder durch Enzyme verflüssigt und durch Alkohol als Aufschlämmung ausgefällt werden, aber es enthält dann noch als Verunreinigungen, z. B. Inulin, Pektinstoffe, Galaktan, Fruktan, Xylan und Araban u. a. M.

So hergestelltes Mannan ist durchaus kein reines Produkt, es musste daher von diesen Begleitstoffen isoliert werden. Es ist dennach also vor allen Dingen wichtig, zur Gewinnung grösserer Mengen von Mannan in reinem Zustande ein Verfahren zu seiner Reinigung auszuarbeiten, insbesondere ein Verfahren des Abbaus durchzuführen und auch in der Frage des Verhältnisse der hydrolysierten Zuckermenge ordnungsgemäss vorzugehen.

Karrer und seine Mitarbeiter fanden, dass das Galaktomannan sich in heissem Wasser, leichter noch in verdünnten Laugen auflöst und durch Fehlinglösung daraus als Kupferverbindung wie Xylan nach Salkowski gefällt werden kann. Setzt man dem pulverisierten Konjakmannan genügend viel Wasser unter Umrühren zu, so erhält man das Produkt in kolloidaler Form. Durch Erhitzung mit Wasser unter Druck (1∼1.5 Atm.) im Autoklaven wird das Konjak-mannan verflüssigt und man gewinnt dann durch Absaugen ein klares, hellgelbes Filtrat, aus dem durch Fehlingsche Lösung das schöh blau gefärbte, voluminöse Kupfermannan ausgefällt wird. Mit alkoholischer Salzsäure zerlegt man diese Aufschlämmung, filtriert das erzeugte Glukomannan ab, wäscht es mit Alkohol und Aether und trocknet es im Vakuum auf Phosphorsäureanhydrid. Das Produkt ist eine feinpulverige, reinweisse Masse, die mit Jod nicht blau färbt und Fehlingsche Lösung nicht mehr reduziert.

Die Ausbeute an Glukomannan und dessen Aschengehalt sind sehr schwankend:

Nr.	Ausbeute	Aschengehalt
1	47.41%	0.40%
2	43.62%	0.28%
3	67.72%	0.10%
4	65.59%	0.19%
5	64.88%	0.45%
6	62.65%	0.32%

Das durch geschriebene Verfahren gewonnene Glukomannan besteht aus Mannose und Glukose, während die Reaktion auf Fruktose negativ ausfiel. Besonders interessant ist hier, dass das Glukomann aus Konjakpulver durch das Auskochen mit Wasser unter Druck im Autoklaven nicht in siner urspringlichen, sondern in einer bereits chemisch veränderten Form erhalten wird, was äusserlich daran zu erkennen ist, dass die Bestandteile des ursprünglichen Konjakmannans Mannose, Glukose und dazu Fruktose oder nur Mannose und Glukose sind. Es ist also eine Verflüssigung durch Enzyme und Bakterien erforderlich, um Ausblicke auf dieses Ziel zu erreichen.

Hierzu verwendet man 10%ige Pankreatin-Lösung mit Toluol, welche dem Konjakpulver zugefügt werden und mit welchen es im Thermostaten auf 35°C erwärmt und in zwei Wochen verflüssigt wird. Die kolloidale Flüssigkeit wurde wie vorher behandelt. Nach dem Trocken ergib sich ebenfalls ein weisses, lockeres Pulver. Die Ausbeute beträgt 57%, der Aschengehalt 0.47%. Darin sehen wir eine weitere, vollkommene Analogie zwischen diesen durch beide Verfahren gewonnene Glukomannanarten.

II. MITTEILUNG. ZUSAMMENSETZUNG DES GLUKOMANNANS DURCH DIE HYDROLYSEN.

(Eingegangen am 14 October 1930.).

Wesentlich grösseres Intersse kommt nun der Zusammensetzung des durch A–bezw. B–Verfahren hergestellten Mannans zu, dem Glukomann oder Fruktoglukomannan, und weiter der Frage, ob sich darin Mannose, Glukose und auch Fruktose und in welchen Verhältnisse vorfinden. Ein geeigneter Weg zur Aufklärung dieser Fragen schien uns nun die quantitatv durchgefürte Hydrolyse zu sein. Zum Verzuckern der höheren Kohlenhydrate sind von verschiedenen Forschern verschiedene Reagentien gesucht worden und als geeignetes Mittel dienen hochkonzentrierte Salzsäure und verdünnte Salz und Schwefelsäure.

Zur Hydrolyse des Glykomannans verwendeten wir Salzsäure in 3 verschiedenen hohen Konzentration, nämlich vom spez. Gew. 1.21, 1.223 und 1.23 in der Kälte. Aus diesen Versuchen geht also hervor, dass die Verzuckerung des Glukomannans in 24 Stunden ihrer Ende erreicht und schwankende Zuckermengen, nämlich zwischen 102∼108% vom Gewicht des Glukomannans (theoritisch 111.1%) ergibt.

Um die Verzuckerung mittels hochkonzentrierter Salzsäure durch die Hydrolyse mit verdünnter Salz–und Schwefelsäure vergleichen zu können, haben wir das Glukomannan durch Erhitzen mit 10%igen, 5%igen und 2%igen Lösungen beider Säuren aufgespalten. In einer Reihe von Versuche zeigte es sich, dass der Aufschluss von Glukomannan durch verdünnte Schwefelsäure sehr langsam verläuft und es erhellt, dass man durch Hydrolyse mit 2%iger Salzsäure während 25 Stunden im Wasserbade die beste Zuckerausbeute erzielt.

Ueber den genauen Sitz der Fruktose wurde früher von Miyake berichtet, doch lässt sich über dieses Glukomannan nichts sicheres ansagen. Der Nachweis der Fruktose fiel in den hier ausgeführten Versuchen nach Seliwanoff mittels der Resorcinprobe negativ aus, dagegen wurde nach Pinoff mit Ammoniummolybdatlösung eine geringe Blaufärbung beobachtet. Wahrscheinlich aber handelte es sich im letzten Falle nun Glukose, da die Intensität der Färbung genau so stark war, als wenn die Lösung nur 0.03 gr. Fruktose enthealten hätte. Am besten hat sich bei Abwesenheit von Fruktose die Bestimmung von Aldose nach Kolthoff bewährt. Aus diesen Versuchen geht hervor, dass man den gesamten hydrolysierten Zucker als Aldose ausgezeichnet jodmetrisch bestimmen kann und dass die Lösung keine Fruktose enthält. Was die Bestandteile des durch das A- bezw. B-Verfahren hergestellten Glukomannans angeht, so sehen wir, dass das Glukomannan bei der Darstellung durch kochendes Wasser unter Druck, gleich wie das Enzyme verflüssigte Glukomannan, so gut wie keine Fruktose enthält.

Da bei der Verzuckerung als Abbauprodukt Mannose und Glukose zu erwarten sind, war es von Wichtigkeit, deren Verhalten in Lösung zu beobachten. Zu diesen Zuwecke war Mannosebestimmung mit Phenylhydrazin und die Feststellung des Drehungsvermögens erforderlich. Die durch Feststellung des Drehungsvermögens bei konz. Säurehydrolyse gewonnenen Ergebnisse stimmten nicht so genau überein, dass wir die Behauptung, das Glukomannan sei aus 2 Mol Mannose und 1 Mol Glukose aufgebaut, aufrecht erhalten könnten. Hier seien die Fälle, bei den Vergleichsproben in denen Glukomannan mit verdünnter Säure verzuckert wurden berücksichtigt; bei solchen Proben entspricht die Genauigkeit der polarisierenden Kraft etwa der Genauigkeit der Zuckerverhältniszahlen. Aus diesen Zahlen ist nun zunächst in Uebereinstimmung mit der Angabe Mayeda's zu folgern, dass das Glukomannan aus 2 Mol Mannose and 1 Mol Glukose aufgebaut ist.

Nach mannigfaltigen Versuchen mit hochkonzentriertem und verdünntem Säureabbau die Zuckerverhältnisse in bezug auf die Menge durch Mannosephenylhydrazonbestimmung wurde befriedigenden Ergebnisse angeführt. Aus dieser Reihe von Vorversuchen ergibt sich, dass das Konjakmannan bei der Hydrolyse 1 Mol Glucose und 1 Mol Mannose lieferte, danach es also als ein Glukomannan aufzufassen wäre. Diese Verhältniszahlen sind, da 1.2 Mol Phenylhydrazin theoretisch genügen, praktisch aber viel weniger hinzugesetzt wurde, nicht brauchbar. (Bei diesen Fällen wurden 1.2 Mol Phenylhydrazin im Becher abgewogen and darauf die 3 fache Menge Eisessig hinzugefügt. Diese Gemisch wurde wieder im Erlenmyerkolben auf die Lösung übergegossen und die Reaktion abgewartet. Hierbei wurde das Phenylhydrazin nicht so gut gemischt und nach dem Abgiessen blieben geringe Mengen in dem Becher zurück). In Hydrolyse soll versucht werden, eine zahlmässige Schlussfolgerung dieser Angabe herzuleiten, wobei die Ermittelung mit 1.5 Mol, 3 Mol und 5 Mol Phenylhydrazinzusatz aufgeführt wurde.

Bei der Betrachtung der Analyseergebnisse fällt zunächst auf, dass die Fehlerquellen und die Ausarbeitung genügend fehlerfreie Bestimmungsverfahen gelöst, und ein deutlisches Bild der Verhältniszahlen erhalten werden kann. Das Polarisationsverfahren bei verdünnter Säurehydrolyse zeigt fast völlige Uebereinstimmung mit dem Phenylhydrazonsverfahren. Danach ist das Glukomannan von Konjak ein polymeres Kohlenhydrat, das aus 2 Mol Mannose und 1 Mol Glukose besteht, d. h. es ist Glukomannan.

(Forstchemische Institut, Kaiserl. Kisuhu Universität).

UNTERSUCHUNG ÜBER DAS ÄTHERISCHE OEL AUS PODOCARPUS MACROPHYLLA, DON.

I. MITTEILUNG. DER BESTANDTEIL DES ÄTHERISCHEN OEL AUS PODOCARPUS MACROPHYLLA, DON

von

Kitsuji Nishida und Hidetake Uota.

(Eingegangen am 26 October 1930).

Podocarpus macrophylla, Don. ist im südlichen Japan, in Formosa und im südlichen China eine etwas häufige Ercheinung. Bei der Destillation des Astes und des Blattes wurde 0.049% ätherisches Oel erhalten. Die Konstanten des nicht unangehem riechenden Oels waren folgende: d_4^{15} 0.9350, $[\alpha]_D^{20}$ $-7.56°$, n_D^{20} 1.50819, Säurezahl eine Spur, Esterzahl nach Acethylierung 26.1.

Das Oel wurde im Vakuum destilliert und die folgenden Fraktionen aufgefangen:

Fraktion	Drück	Temperatur	%	Farbe	d_4^{15}	n_D^{20}	$[\alpha]_D^{20}$
I	6 mm	42~44°	4.00	farblose	0.8555	1.46847	+28°02
II	5 〃	46~48°	4.13	〃	0.8513	1.47131	+46°40
III	5 〃	56~60°	2.53	hellgelb	0.8572	1.47040	+57°58
IV	3 〃	68~70°	0.70	〃	——	1.48613	+27°60
V	3 〃	90~94°	1.07	〃	——	1.49342	+ 1°40
VI	3 〃	104~105°	1.31	〃	——	1.49492	− 5°72
VII	4 〃	107~110°	9.05	〃	0.9110	1.49596	− 7°60
VIII	4 〃	113~115°	2.93	〃	0.9148	1.40417	− 4°92
IX	3.5 〃	110~112°	2.23	〃	0.9170	1.50096	− 5°16
X	3.5 〃	123~125°	3.13	〃	0.9253	1.50591	− 9°20
XI	3.5 〃	138~140°	5.88	〃	0.9400	1.51062	− 9°23
XII	3 〃	153~169°	47,33	〃	0.9710	1.52201	−13°32
XIII	3 〃	173~177°	9.34	〃	0.9873	1.52738	−40°92
Rückstand				rotbraun			

Aus verseiftem Oel.

I′	4.5 mm	39～40°5	7.10	farblose	0.8523	1.471016	+33°40
II′	5 〃	45～47°	3.34	〃	0.	1.472094	+54°64
III′	5 〃	99～101°	5.74	hellgelb	0.9040	1.493420	− 1°00
IV′	4 〃	104～106°	5.83	〃	0.9099	1.498384	− 9°40
V′	4.5 〃	109～120°	8.08	〃	0.9150	1.501606	−10°60
VI′	3 〃	138～140°	10.24	〃	0.9483	1.514000	−11°52
VII′	2.5 〃	156～157°	17.45	〃	0.9687	1.520818	−20°60
VIII′	2.5 〃	157～158°	11.09	〃	0.9715	1.522624	−24°00
IX′	2.2 〃	162～164°	13.93	dünntesten	0.9764	1.523815	−32°40
X′	2.2 〃	164～165°	11.10	hellgelb	0.9828	——	——
XI′	Rückstand		1.03	dünkelbraun	——	——	——

Die niedrigst siedenden Anteile des Oels (I, II und I′ Fraktion $[\alpha]_D^{20}$ (+)46°～(+)28°) gab mit Amylnitrit und Salzsäure ein Nitrosochlorid (Smp. 103°C). Es war auch möglich, α–Pinennitrosochlorid durch Umsetzung mit Piperidin zu Pinennitrolpiperidin vom Smp. 117～118°C nachzuweisen. Bei der Prüfung der II und III Fraktion auf Camphen mit Eisessig-Schwefelsäure wurde Isobornylacetat erhalten, das bei der Verseifung Isoborneol ergab. Fenchen war in der I Fraktion nicht enthalten. β–Pinen liess sich in dem Anteil von Smp. 56～70°C (3～5 mm) aus der III und IV Fraktion mit Sicherheit nachweisen. Es entstanden bei der Oxydation mit alkalischen Permanganat gläzende Blättchen eines nopinsaures Natrium, die leicht Nopinsäure (Smp. 121 − 122°C) darstellen. V und III′ Fraktion gaben nur eine Abscheidung eines bei 118°C schmelzenden Cadinenchlorhydrat. Dagegen waren diese Fraktionen nicht caryophyllen und sylvestrenhaltig. Zwei neue Dipentenen aus XI～XIII and VI～X′ Fraktion wurden in II Mitteilung erklärt.

(Forstchem. Institut, Kaiserl. Kiushu Universität).

ON GLUCONIC FERMENTATION PART I.*

ON BACTERIUM HOSHIGAKI VAR ROSEA NOV. SPEC.

By

TEIZO TAKAHASHI and TOSHINOBU ASAI.

(Received 18 th., Nobember, 1930.)

Boutroux's⁽¹⁾ memoir on gluconic acid formation as a fermentation product

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* This was already published in "Nihon Nōgei Kwagaku Kwaishi" Vol. VI. Vo. 3. March. 1930. (Japanese language).

offered in 1880, cast us very interesting and important problem; although the occurrence of gluconic acid in the product of his lactic acid fermentation was quite beyond the scope of his expectation; viz. the lactic acid. Later, Brown(2) confirmed more satisfactory in bacteriological point of view the formation of this acid by his Bact. aceti and xylinum. Bact. Kützingianum and Bact. Pasteurianum behave in the same way after Seifert(3). Almost all species of Henneberg's(4) acetic bacteria and in likewise Termobacterium aceti produce gluconic acid from glucose after Zeidler(5). Hoyer's(6) two varieties of Bact. rancens viz. var. Zythi and var muciparum and Acetobacter melanogenum after Beijerinck(7), as well as Miyaji's(8) Bact. acetigenum var. Bact, rancens var. and Bact. vini acetati var. and a variety of Bact. industrium of Yagi and Hashitani(9) belong to the same category of acetic bacteria.

Recently, S. Hermann(10) isolated from Japanese Kombucha (Algae-tea) special bacteria named Bact. gluconicum, which producing an abundant quantity of gluconic acid. The percentage of the acid formed from glucose attained in his case to about 80, excelling all instances prior to him.

R. Falk and S. R. Kapurs'(11) observation gave some light upon the nature of the gluconic fermentation of mould. They concluded that Asp. niger, Asp. cinnamomens, Asp. fuscus and other fungi causing citric fermentation produce gluconic acid. About Asp. niger K. Bernhaner(12) mentions the same fact, and C. Wehmer(13) found the same acid in the fermentation product of Asp. fumaricus. K. Sakaguchi's(14) observation on the varieties of Asp. oryzae confirmed the occurrence of this acid in their products by the great majorities. The same author gave evidence on the production of 50% of the acid stated by Asp. wentii.

The fungus of penicillium group also behave to glucose in the same way, especially Penicillium luteum has been shown to form this acid after O. E. May, Thom and M. B. Church(15). The variety of the said fungus viz., Pen. luteum purpurogenum produce this acid up to 56~57% or even to 64.7% if there were associated special mineral salts to the nutrient medium after O. E. May and H. I. Herrick(16).

In regard to the products of Rhizopus species one of us (T. T)(17) with his co-worker K. Sakaguchi affirmed this acid associated many other organic acids.

Recently, the authors, in the long (about four months) culture of Absidia viz., Ab. spinosa as well as Ab. septata confirmed the occurrence of gluconic acid, especially by the former species amounting to 60% as its yield. The details will be expected to communicate in a short while.

S. Hermann's(18) observation quoted above, stimulated us to undertake in the similar way about "Kombu-cha" as well as to follow research about

"Hoshigaki" or dried persimmon with anticipation that an analogous bacteria should be found from the latter material. Unfortunately, we could not find out any acetic bacteria from the former until now inspite of many samples were examined. On the other hand, from dried persimmon four varieties of acetic bacteria, one lactic bacteria, two mycoderma yeasts and one mould were isolated.

All of these acetic bacteria are quite new species and moreover produce gluconic acid from glucose amounting to 80∼100%, especially one of them specifies itself in producing a beautiful red colouring matter when it was cultured in medium associated with $CaCO_3$. In this paper, inter alia, this special one is to be described, the other three species will be published before long.

Experimental Part.

The fermentation products of "Hoshigaki"

To prepare the fermenting mash, 20 g. of "Hoshigaki" was chopped into small pieces, which were introduced into a sterilised flask containing 200 c.c. of sterilised water. The flask was held in an incubater at 28∼33°C during ten days.

In the begining the evolution of the gaseous matter were perceived, thence followed the formation of the film on the surface of the fluid associating the brown deposit at the bottom of it. At the end of incubation there was perceived considerable amount of acid(1), which attained in terms of n/10 NaOH solution up to 12.3 c.c. when 10 c.c. of the fermented fluid was examined.

By subjecting the fermented fluid to the steam distillation, only a trace of volatile acid were perceived, suggesting the predominancy of some non-volatile acid in it.

The latter acid was isolated in the form of salt as crystallised mass, seeming like cauliflower, from the residual fluid of the steam distillation, provided the neutralisation with $CaCO_3$ and condencing of it. The salt was difficultly soluble in the cold water but easily when hot and insoluble in alcohol.

Identification of the acid.

(1) It gave the characteristic blue colour reaction to β–naphthol(19) in presence of H_2SO_4.

(2) Its Ca–salt is easily soluble in hot water and precipitated by alcohol. On standing the solution in dilute (50%) alcohol, there comes force beautiful needle shaped crystalls of Ca–gluconate.

(3) The water of crystallisation of barium salt seems to be just one molecule thus:——

---

(1) The acidity of original fluid, in terms of n/10 NaOH solution, was 0.1 c.c. for 10 c.c. of it.

Subst. taken	0.5099 g.
After drying at 110∼115°C	0.4919 g.
Difference.	0.0180 g.

$\therefore$ % of $H_2O=3.53$

Calc. as one molecule of water 3.30%.

(4) The contents of barium in the salt agree with that of gluconic salt thus :——

Subst. taken (water free)			0.4919 g.
$BaSO_4$ weighed	0.2168 g.	$\therefore$	Ba. 0.1276.
	Found.		25.92% Ba.
	Calcul.		26.04% Ba.

(5) The melting point of its phenylhydrazid was 196∼200°C, coinsiding with that given in the literature. The percentage of nitrogen of the same was :——

Subst. taken	0.5022 g.	N. gained	0.04934 g.
		Found.	9.83% N.
		Calcul.	9.79% N.

(6) The specific rotatory power was +5.91° thus :——

Subst. taken (Ca-salt) 1.1325 g. water 10 c.c.

Temp. 21°C. $l=1$ d. m.

$\alpha=+0.67^\circ$

$$\therefore\ [\alpha]_D^{21}=\frac{\alpha\times100}{d\times l}=\frac{0.670\times100}{11.3225\times1}=+5.91^\circ$$

For the reference,

One of us (T. T) and Sakaguchi's(20) result.	$[\alpha]_D^{17}=+5.73^\circ$ (C=3.227)
Herzfeld(21)	$[\alpha]_D=+5.8\sim+5.94^\circ$, (C=Ca 2.)
Bertrand(22)	$[\alpha]_D^{20}=+6.13^\circ$, (C=5.0)
Fischer(23)	$[\alpha]_D=+6.66^\circ$

Bacterium Hoshigaki var rosea. nov. Spec.

This bacteria was isolated from a fermenting mash prepared as the mixture of chopped "Hoshigaki" and sterile water in flask, which was sterilized prior to the introduction of the materials. It forms acetic acid in alcohol fluid but gluconic acid makes its predominancy in case of the nutrient medium containing sugars and devoid of alcohol. It produces *rose red colour* in medium containing $CaCO_3$ and from this character we propose to call this bacterium, *Bacterium Hoshigaki var rosea.*

Form and Size :—— Short rods, 1.5∼1.8 μ long, about 0.7∼0.9 μ thick. Generally single; at most in pairs; often in chains. Non-motile. It has vigorous tendency to form involution forms at room temperature. The pellicle on fluid media has no reaction of sterch or cellulose.

Plate cultures. (Incubated at 26∼28°C.)

Koji-Extract Agar :—— After two days appear as small round granular shining colonies, with elevation at the center, which altered to brown colour. Magnified to 150 times: Smooth border with light brown colour and the center is coloured dark brown with compact granular surface.

Wort Agar:—— Round milky white colonies after two days. The center of them gradually alters to brown in contrast to yellowish brown periphery.

Glucose Sake' Agar:—— Round, milky white granular colonies with elevation of center and smooth periphery after two days. Colour of center changes from yellowish brown to brown, when periphery acquires yellowish white tint.

"Hoshigaki"–Extract Agar:—— Round, milky white granular colonies after two days. Gradually, the center acquires yellowish brown colour and periphery gray white.

Agar Stab. (Incubated at 26∼28°C.)

Media.	Remarks.	Form and size of cells.	Involution form.
"Koji"–Ext. Agar. Stab:	Feable growth. Surface growth: Gray white, shining flat growth after two days. Later, acquiring brown colour going to lusterless. Medium at stab mouth changes to brown.	Short red, single or in pairs. 0.7∼0.9μ× 0.7∼1.45μ.	none.
Wort Agar. Stab:	Almost no growth. Surface growth: Flat growth with paste-like luster after two days. Later it aquires from gray white to brown colour. Medium under surface growth acquires dark brown culour.	Short rod.	Occurs Vely seldom
Glucose Yeast Water Agr. Stab:	Feable growth like entangled hair near the mouth, coloured reddish brown which changes to light brown in deep part. Suface growth: White round growth with pasty luster after two days. Later it becoms flat.	Short rod.	Frequently occurs. 6∼10μ long.
Glucose Sake' Agar. Stab:	Feable growth. Surface growth: Milky white round growth after two days. Later acquires fine light rose colour accompanying the elevation of periphenry. Medium changes somewhat brown.	Short rcd, single, pair or in chain. 0.5∼1.45 μ× 0.5∼1.8 μ	None.
Bouillon Agar. Stab:	Almost no growth in stab canal. Strface growth: Gray white pasty flat growth.		
Yeast Water Agar. Stab:	Almost no growth in stab canal. Surface growth: Almost transparent irregular growth with luster around the mouth of stab.		
"Hoshigaki" Extract Agar. Stab:	Feable growth chiefly at upper part of stab canal. Surface growth: Gray white flat colony with luster. The central part changed to brown.	Short rod.	

Agar Streak. (Incubated at 26∼28°C.)

Media.	Remarks.	Cell form and size.	Involution form.
"Koji"–ext. Agr:	Grayish-white, shining growth, with pasty appearance, showing hairy outgrowths. Later it colours purple brown to brown. The water of condensation is turbid with film and deposit.	Short rod, single or pairs. Rarely in chains. 0.7∼0.9μ×0.7∼1.45μ.	None.
Wort Agar:	Generally same as above, with exception of dense brown colour which degradate to light brown on margin part by this medium. Beside no hairy out-growth appears in this medium.	Same as above with size of 0.7∼0.9μ ×0.7∼1.1μ.	Occurs very seldom with size of 2μ×7μ.
Glucose Yeat Water Agar:	Same as above with diffence of wavy periphery. The water of condensation is turbid with light brown deposit.	Same as above with size of 0.5∼0·7μ ×0.7∼0.9μ.	None.

Glucose Saké Agar :	Same as above except the yellowish brown coloured marginal elevation along innoculation streak. The water of condensation is turbid with gray white deposit.	Short rod, single or pairs. Seldom in chains with size of 0.5∼0.9μ× 0.5∼1.45μ.	None.
Bouillon Agar :	The growth remains limited to the inoculation streak, although it has a pasty consistency.	Short rod, single or pairs, with size of 0.5∼0.9μ× 0.5∼1.45μ.	Swollen cells with 1.5× 2.0∼3.0μ.
Yeast Water Agar :	Feable growth along innoculated streak. The colour is light brown.	Same form and size as above, but chain forms are found.	Swollen cells with size of 3μ×4μ.
"Hoshigaki"-Ext. Agar :	Pasty growth along inoculation streak. The brown colour is dense at central part in contrast to light brown periphery.	Same form and size as above.	None.

The culture in fluid media. (Incubated at 26∼28°C).

Media.	The growth.	Form and size of cells.	Involution form.	Remarks.
"Koji"-Extract :	The fluid becomes turbid after two days. Thin film begins to appear after three days, and gradually ascends to the wall of tube.	Short rod single, pairs or in chain with size of 0.9× 0.9∼1.45μ.	It occurs seldom as long thread, 1×2∼4μ or 1×40μ.	
Wort :	The fluid turns turbid after three days, with film on surface. The peposit turns reddish brown after long.	Same as above. 0.7∼0.9 × 0.7∼1.45μ.	Occurs very seldom, 1.2×1.5μ.	The fluid turns red after 5 days.
"Hoshigaki"-Extract :	The fluid turns turbid after three days, with very thin film. A trace of bacteria ring was observed. The deposit and bacteria ring change to yellowish brown at the end of the cultivation.	Same as above, but with different sizes as such, 0.9× 1.45∼1.8μ.	Occurs very rare.	
Hopped Wort :	The film is formed over turbid fluid after two days. It ascend mor or less to the wall of vessel, giving rise the formation of bacteria ring, which changes to brown as deposit alters to same colour.	Same as above, except the size of 0.7∼ 0.9μ×0.7∼ 1.8μ.	Occurs seldom as curved therad.	The fluid turns red colour after five days.
Alcohol free Beer :	Turbid after 24 hours, with very thin film. Later, bacteria ring and deposite colours brown.	Same as in wort.	Occurs very rare.	
Yeast Water :	Somewhat turbid after two days with coarse bacteria ring.	Same as hopped wort adding chainform by this medium.	None.	
Glucose Yeast Water :	Bacteria ring formed after two days. After three days, film appear under which the fluid turns turbid. The deposit and ring colour brown after long.	Same as in wort.	Thread cells are observed, sttaining to 30μ long.	
Bouillon :	The fluid turns somewhat turbid after two days associated with bacteria ring. Film appears after five days.	Short rod, single, pairs or rarely in chains, with the size of 0.9μ× 1.45∼1.62.μ	Occurs rarely with the length of 12∼20μ.	

Alcohol free Glucose Saké: The fluid turns turbid with film over the surface after two days. The deposit and bacterta ring gain brown colour.	Same as in wort.	Almost none.	After four days the fluid gains fine red colour.
Glucose Saké: (Alcohol Ca. 4%). Bacteria ring begins to form after two days. The fluid turns somewhat turbid and the film ascends to the wall of vessel. Deposit and bacteria ring colour brown.	Short rod, single with the size of 0.9μ×0.9~1.8μ.	Occurs abundant as long thread with swollen bulb. Some attain to 50μ length	The fluid gains red colour.
Beer: (Alcohol Ca. 4%). The fluid turns turbid with thin film which ascends somewhat, after three days.	Same as in yaest water or hopped wort, wanting chain form.	Occurs as thread or swollen thread with the size of 1.5~2.0μ×2.0μ.	
Alcohol (3%) "Koji"-Ext: Same as in beer.	Same as in glucose yeast-water.	Occurs as short long or swollen thread, attaining to 100μ in the latter case.	
Alcohol (3%) Wort: Same as in beer but addition of brown coloured bacteria ring.	Same as in glucose yeast-water, wanting chain form.	Occurs seldom, as curved thread.	
Alcohol (3%) "Hoshigaki"-Ext.: Same as in beer.	Same as in alcohol wort.	Occurs as long thread.	
Alcohol (3%) Glucose-Yeast-Water: Same as in alcohol wort.	Same as in yeast-water, adding chain form.	Occurs as swollen or curved short thread.	
Alcohol (3%) Bouillon: The fluid alters turbid after three days.			
Führmann's solution: No growth.			
Beijerinck's solution: No growth.			
Pasteur's solution: No growth.			
Janke's solution: No growth.			
Henneberg's solution: (Asparagin). No growth.			
Same but pepton: Bacteria ring at the wall of vessel after two days. Turbidity appeared with white spotted film.			
Same, but Urea: No growth.			
Henneberg's II. solution: (devoid of alcohol). No growth.			

N. B. Füermann's solution:(24) Am-phosphate 0.2 g., Am-sulphate 0.1 g., Acid K-phosphate 0.1 g., Mg-sulphate 0.1 g., Distil. water 800 c.c. glucose 5%.

Beijerinck's solution :(25). Am–phosphate 0.5 g., KCl 0.1 g., Distil. water 1000 c.c. Alcohol 3%.

Pasteur's solution :(26). Am–phosphate 0.2 g., K–phosphate 0.1 g., Ca–phosphate 0.1 g., Acetic acid glacial 12.5 g., Alcohol 22.5 g., Distil, water 1000 c.c.

Henneberg's II solution :(27). Am–sulphate 0.3 g., Acid K–phosphate 0.3 g., Mg–sulphate 0.2 g., Glucose 5 g., Alcohol 2 g., Distil. water 100 c.c.

Janke's solution :(28). Am–phosphate 1 g., Acid–K–phosphate 0.4 g., Mg–sulphate 0.4 g., Glycerin 5 c.c., Succinic acid 1 g., Distil. water 1000 c.c.

Henneberg's solution :(29). Peptone (or Asparagin, Urea) 10 g., Acid–K–phosphate 1 g., Ca–phosphate 1 g., Mg–sulphate 1 g., Glucose 50 g., Distil. water 1000 c.c.

Acid formation from carbohydrates and alcohols.

(Incubated at 26~28°C during 17 days.)

The medium used was yeast water, containing 2% of alcohol or carbohyhydrate. Control yeast water seeded with bacteria, needed 1.1 c.c. of n/10 NaOH solution to neutralize 10 c.c. of medium after nine days and increased to 1.15 c.c. of the former after seventeen days.

As the result the production of acid was ascertained in the cases of arabinose, glucose, galactose, glycerin, mannit, ethyl alcohol, propyl alcohol, as well as from saccharose, maltose, lactose, dextrin, inulin, glycogen, sorbit, isodulcit, and methyl alcohol although only a trace, but starch and raffinose could not stand for as the source of acid production.

The details are tabulated below :——

Carbohydrates or alcohols.	Acidity (c.c. of n/10 NaOH. for 10 c c. of the culture).		Remarks.
	After nine days	After seventeen days.	
Arabinose.	4.5	4·9	
Glucose.	11.1	12.1	
Fructose.	1.45	1.5	Faint growth.
Galactose.	5.0	5.0	
Saccharose.	1.1	1.3	Slight growth. Medium dose not reduce Fehling's solution.
Maltose.	1.2	1.3	Slight growth as shown in saccharose case.
Raffinose.	1.15	1.15	Faint growth.
Dextrine.	1.2	1.35	
Starch.	1.1	1.1	Very feable growth without film formation.
Glycogen.	1.3	1.3	Almost no growth.
Inulin.	1.3	1.3	Faint growth.
Glycerin.	1.8	2.0	Medium reduces Fehling's solution when hot.

Mannit.	2.05	1.9	Do.
Sorbit.	1.3	1.4	Do.
Isodulcit.	1.0	1.3	Do.
Methyl alcohol.	1.3	1.3	
Ethyl alcohol.	20.4	23.9	
Prophyl alcohol.	20.8	22.5	

Registance against glucose and its optimum concentration for the growth.

The yeast water(2) containing glucose, varying from 5 to 50%, was used for ascertaining the registant power of this bacillus against glucose and optimum concentration for its growth of the latter.

It was incubated at 26∼28°C during twenty days and as the result gained about 40% of glucose as the highest limit of concentration for the growth, in as much as we could not find out any growth in 55% of the same.

The optimum concentration of glucose was 10%, although the highest concentration of gluconic acid gained was 17.27%, which was found in the solution containing 20% of glucose.

The table below shows the details :——

% of glucose.	After					Acidity(3) after 20 days, c.c. of n/10 NaOH	Gluconic a. %.
	2 days.	4 days.	9 days.	11 days.	20 days.		
5.0	Fluid turbid with yellowish milky white bacteria ring and heavy yellow deposit.	Same as before, but with brown bacteria ring.			Deposit altered to brown.	28.0	5.48
10.0	Same as above.	Deposite increased.			Deposit altered to brown.	52.8	10.34
20.0	Little turbid.	Milky white bacteria ring, with deposit.	deposit increased.		Deposit altered to yellowish brown.	**88 0**	**17 27**
30.0	——	Little turbid.	Turbid increased with bacteria ring.		Turbid added yellowish brown deposit.	81.2	15.91
35.0	——	——	——	Little turbid.	Heavy turbid with milky white bacteria ring and yellow deposite.	38.2	7.48
40.0	——	——	——	Little turbid.	Added slight deposit.	9.2	1.80
45.0	——	——	——	——	——	——	——

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(2) Its original acidity corresponds to 0.6 c c. of n/10 NaOH solution for 10 c.c.

(3) The acidity shown below was obtained from the difference of the acidity after cultivation and that of the original yeast water.

Registance against acetic acid.

For the ascertion of the registance against acetic acid of this bacillus, beer(4) was taken as original medium and to which acetic acid was added in the desired percentage, varying from 0.5 to 2.5%. The bacillus could not show any growth in beer which added 2.0% of the stated acid. The details are to be seen in the table below :——

% of acetic acid added.	After					Acidity shown by c.c. of n/10 NaOH	
	3days	4days	8days	13days	19days	After growth	Before growth
0	Fluid turbid, thin film with white ring.	——	——	——	——	44.8	1.2
0.5	No growth.	Little turbid with thin film and ring.	——	——	——	46.8	8.3
1.0	No growth.	No growth.	Little turbid with thin film and ring.	——	——	42.8	16.6
1.5	No growth.	No growth.	No growth.	Very faint turbid with slight ring.	——	45.6	24.9
2.0	No growth.	——	——	——	——	46.8	38.2

Registance against ethyl alcohol and the maximum yield of acetic acid.

To determine the registance against ethyl alcohol of this bacillus, yeast water(5) was used as the medium, adding ethyl alcohol in the desired percentage viz : 1.0∼8.0%. All the cultures were incubated at 26∼28°C during 20 days. In the medium containing 7% of alcohol, this bacillus could not show any growth and any production of acetic acid. The optimum concentration of ethyl alconol for the production of the acid was 3 vol. %, gaining 1.87% acetic acid.

The table below shows the details :——

% of alcohol added.	After						Acidity(6) gained after 20 days. c c. of n/10NaOH	% of acetic acid.
	2days	3days	6days	9days	12days	20days		
0	Little turbid with very faint film.	——	——	——	Turbid added bacteria ring and yellow white deposit.	As before	1.3	——

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(4) This beer contained 4 vol. % of alcohol and the acidity as such ;— needed 1.2 c.c. of n/10 NaOH solution to neutralize 10 c.c. of the beer.

(5) The acidity of the original medium was 0.6 c.c. n/10 NaOH for 10 c.c. of the medium.

(6) Acidity quoted below is the difference of the acidity of culture and that of the control.

1.0	As above with addition of yellow powderly deposit.	Film ascend to wall of the vessel.	——	——	Heavy turbidity	As before	21.8	1.30
3.0	Little turbid with very faint film.	——	bacteria ring appear.	——	Turbid increased with slight deposit.	As before but added deposit.	31.2	1.87
4.0	As above.	——	As above.	——	As above.	As before but added deposit.	21.4	1.28
5.0	No growth.	Trace of turbid.	——	——	Turbid added the deposit.	As before but added deposit.	21.4	1.28
6.0	No growth.	——	——	Trace of the turbid	Increased turbid.	Deposit only	1.5	Trace.
7.0	No growth.	——	——	——	Trace of turbid.	Trace of deposit.	——	——
8.0	No growth.	——	——	——	——	——	——	——

Registance against common salt.

(Incubated at 26～28°C during 22 days.)

"Koji"–extract⁽⁷⁾ (12°B) as culture medium containing the desired concentration of common salt was used for the determination of the registance of this bacillus against common salt, varying from 0.5 to 2.5%. At the end of the cultivation, the limit of the contents of common salt for the growth was found to lie between 1.5～2.0%. In general, common salt in the medium always plays some role for the deterioration of the growth of this bacillus.

The details are tabulated below :——

% of NaCl added.	After					Acidity⁽⁸⁾ after 22 days :—— c.c. of n/10 NaOH. for 10 c.c.
	1day	2days	4days	10days	22days	
0	Very thin film.	Film ascending with turbid.	As before adding yellow brown ring.	Ring altered to brown.	——	46.6
0.5	Trace of bacteria ring.	Added trace of turbid.	Added some deposit.	Doposit altered to brown.	——	29.0
1.0	No growth.	Faint ascending film.	——	Added milky white ring and trace of brown deposit.	——	19.0
1.5	No growth.	——	——	Faint film, with brown deposit.	——	11.0
2.0	No growth.	——	——	——	——	4.0

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(7) The acidity of the medium was 2.2 c.c. of n/10 NaOH for 10 c.c. of it.

(8) Acidity is given from the difference of c.c. of n/10 NaOH for 10 c.c. after 22 days and that of the original solution.

Temperature for the growth, acid production and death.

The optimum temperature for the growth was 30~35°C and at 40~41°C there was no growth although it makes very slow and slight growth at 10~15°C.

The death of the bacillus comes force when it is treated five minutes at 50°C in water.

The optimum temperature for the acid production lies between 26~28°C as it is shown in the following table:— (The culture of 10% glucose yeast water).

Temp. C.	After One day.	 2 days.	 4 days.	Acidity; c c. of n/10 NaOH to neutralize 10 c.c. of solution
10~15°	–	–	∓	10.2
26~28°	+	++	++	**51 0**
30~31°	+	++	++	47.6
36°	++	++	++	31.8
40~41°	–	–	–	–

Remarks: + and—denote the presence and absence of the acid. Acidity in the table means the difference of the acidity of culture and that of original medium.

The maximum yield of gluconic acid.

To determine the maximum yield of gluconic acid, yeast water containing 10% of glucose (Merck) was used and incubated at 26~28°C. The acid attained, after 10 or 18 days, to the maximum yield as recorded in the following table.

Days.	Acidity c.c n/10 NaOH	Gluconic acid %	Days.	Acidity c.c. n/10 NaOH	Gluconic acid %
3	14.7	2.88	13	51.2	10.03
4	25.8	5.05	18	52.4	10.11
6	45.8	8.97	22	51.6	10.11
8	50.6	10.91	30	51.2	10.03
10	51.8	10.15	50	51.8	10.15

Is it possible the oxydation of acetic acid by this bacillus.?

To yeast water 3% of ethyl alcohol was added and was seeded with the bacillus prior to the incubation at 26~28°C. The other medium which was added 3% of acetic acid instead of ethyl alcohol, was used for the determination of the loss by mere evaporation. In either case, there was no loss by oxydation of ethyl alcohol, inasmuch as it was under the limit of the loss by evaporation, as shown in the table below:——

Duration of cultivation. days.	Acidity of each 10 c.c. of the medium Alcohol added prior to the seeding.	 Added acetic acid and no infection of bacillus.
5	1.4	37.2

8	1.4	34.8
12	26.4	35.8
15	25.8	——
20	26.4	34.0
30	25.0	——
40	21.2	33.0

The production of gluconic acid by authors bacilli and other bacilli already known.

To determine the maximum yield of gluconic acid by our bacilli glucose (10% Merck)—yeast water was used. The cultures were incubated at 26∼28°C during 14 days to obtain their maximum yield.

Bacterium Hoshigaki var. rosea n. sp. gave the most highest yield of gluconic acid among bacilli already known, although our new bacillus viz: Bact. industrium var Hoshigaki excelled over in its yield. The experimental data are summarised below:——

Bacterium.	Acidity for 10 c.c. of medium expressed by c.c. of n/10 NaOH
Bact. industrium v. Hoshigaki: n. sp.(9)	52. (after 6 days).
Bact. Hoshigaki v. rosea n. sp.	51.2 (after 14 days).
Bact. gluconicum(30)	45.4
Author's bacterium C.	43.0 (after 14 days).
Bact. industrium(10)	42.8
Author's bacterium B.	33.0 (after 14 days).
Bact. oxydans.(10)	32.2
Bacillus of Yagi and Hashitani.(31)	28.0 (after 20 days).
Bact. aceti.(10)	13.4
Bact. acetigenum.(10)	9.8
Thermobact. aceti.(10)	8.0
Bact. xylinum.(10)	6.4
Bact. acetosum.(10)	5.0
Bact. Pasteurianum.(10)	1.2
Bact. Kützingianum.(10)	0.8

Affinities and characters of Bact. Hoshigaki var rosea n. spec.

This bacterium is nonmotile and its film in fluid medium has no reaction of starch and cellulose. It cause a turbidity in fluid medium but has no power of aciditying maltose. From these points stated it stands very near to Bact. acetosum, Bact. rancens, Bact. ascendens or Bact. gluconic Hermann and by the property of producing abundant quantity of gluconic acid, it should be classed in the same category to Bact. industrium, Bact. oxydans, Bact. gluconicum Hermann and bacillus of Yagi and Hoshitani. Although with such properties, as mentioned, analogous to bacilli already known, yet we have many other specific properties characteristic to identify this as quite new one as tabulated in next page. Among the table we find *a red colour production of this bacillus in wort or " Saké "* and the same in all media when they contain Ca-carbonate in addition to them. This *colour production* is a quite specific properties of this one and none of acetic bacilli already known, has ever do the same. The yield of gluconic acid from glucose is most highest among bacteria ever known.

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(9) On the nature of this bacillus and Bact. B et C. the report will be followed soon.

(10) All bacterium marked with, are quoted from Henneberg's Gährings bakteriologie.

The concentration of gluconic acid obtainable in medium by this bacillus.

Two kinds of the medium were used viz.——

(1)	Glucose (Merck)	8 g.	
	Hoshigaki (3%) ext.	100 c.c.	This contained 9.850 g. glucose in 100 c.c.
(2)	Glucose (Merck)	10 g.	
	Yeast water	100 c c.	

The culture held at room tep. (Ca. 25°C) during 14 days.

In the second medium, the accumilation of gluconic acid attained already to 79.8% of the total glucose after 6 days from the begining and after 14 days it increased to 97.8% viz. 98.6% of the calculated yield.

The first medium held good as favorable for this organism, as shown in the table below. The yield attained about 90% of the total glucose.

Medium.	Duration of cultivation. Days.	Glucose. oxydised. g.	Volatile acid c.c. of n/10 NaOH	Gluconic acid.	$\frac{\text{Gluco. Acid}}{\text{Tot. Glucose.}} \times 100$	$\frac{\text{Glu. a.}}{\text{Glucose oxydised.}} \times 100$	Cal %
Hoshigaki Ext. +glucose.	13	8.76	Trace	8.903	89.0	101.6	99.1
Yeast-water glucose.	6	7.85	Trace	7.983	79.8	101.7	93.5
Same.	10	8.68	2.1	8.874	88.7	102.2	93.9
Same.	14	9.118	4.0	9.782	97.8	107.2	98.6

N. B. a.) Glucose was determined by Bertrand's method. b.) Volatile acid The steam distillation was applied to the filtered medium and the distillate was titrated with n/10 NaOH solution associated phenolphthalein as indicater. c.) Gluconic acid : The residue of steam distillation was neutralized with Ca-carbonate and evaporated to a thick mass, prior to the development of crystalls of Ca-gluconate, which was washed by spirit (80%) to remove adhered glucose.

	Bact. Hoshigaki var rosea. n. sp.	Bact. acetosum	Bact. ascendens.	Bact. gluconicum. Hermann	Bact. industrium.	Bact. oxydans.	Bacillus of Yagi et Hashitani.
Nature of film.	Very thin film. like powder. Bact. ring ascend to wall of vessel.	White flat film, which forms foulds. Trace of ascending growth.	Like Bact. Hoshigaki.	No film. occurs.	Thick pasty film, which ascend to the wall of vessel.	Thin film, ascend to the wall of vessel.	Fragmental film.
Cell form and its size.	Majority are single or pair but chain form seldom occurs, 0.5~0.9μ.×0.5~1.45μ.	Long chain form 0.4~0.8μ.×1.0μ.	Single or pair Chain form occurs seldom. 0.7 ~0.9μ.×0.9~1.4μ.	Diplococcus.	Irregular aggregates or short chain. 0.8~1.2μ ×1.6~1.8μ.	Single and its form indefinite Long chain. 0.8~1.2μ.× 2.4~2.8μ.	Short bacillus, 0.8~1.0×2.3 ×3.0μ.
Involution form.	Involution from with swollen. bulb. some short or long or curved. Gigantic cells.	Spindle shaped threads.	Thread with swollen bnlb, are branched Branch is longer than the case of oxydans.		Occurs seldom.	Threads with swollen bulbs and branches.	Occurs seldom.
Starch and cellose reaction of film.	–	–	–	–	–	–	–
Motility	–	–	–	–	–	–	–
Registance limit againt glucose (%).	40	45	35	50	50	35	35
The same againt ethyl alcohol (%).	6~7	11	12	9 vol %	6~7	7	6
The same against NaCl (%).	1.5~2.0	above 3			1~3	0.3	
The same against acetic acid (%).	1.5~2.0	above 2	2~3		1.5~2.0	1.5~2.0	
Maximum concentration of acetic acid formed %.	1.8	6.6	9.1	1.6	2.7	2.0	0.38
Yield of propyric acid formed (2.0% propyl alcohol.)	1.66	1.8	1.9		1.46		
Maximum concentration of gluconic acid produced %.	17.2	4.6			16.6	8.0	12~13
Optimum temp. for growth. C.	30~35°C	28°	34°		23°	12~21°	
Oxydation of acetic acid.	±	+	+		–	–	
Production of colouring mater.	Red colouring matter is formed, in wort, ‘Sak’e or any nutrient medium containing Ca-carbonate.	–	–	–	–	–	–
Assimilation of Am-salts	–	+	+	+			
Acid formation from Arabinose	+	–	–	+	+	+	+
Glucose.	+	+	+	+	+	+	+
Galactose.	+	+	–	+	+	+	+
Fructose.	(+)	–	–	+	+	+	+
Saccharose.	–	–	–	+	+	+	+
Maltose.	–	–	–	–	+	+	(+)
Lactose.	–	–	–	–	+	(+)	+
Raffinose.	–	–	–	+	+	+	
Dextrine.	–	–	–	(+)	+	+	+
Starch.	–	–	–	–	+	–	(+)
Inulin.	–	–		–	–	–	
Methyl-alcohol.	–	–	–	–	(+)	–	–
Ethyl-alcohol.	+	+	+	+	+	+	+
Propyl-alcohol.	+	+	+	+	+	+	
Glycerine.	+	–	–	+	+	+	–
Mannite.	+	–	–	(+)	+	+	+
Sorbite.	–						

The yield of gluconic acid and the nature of nitrogen source.

For gluconic acid fermentation of Bact. Hoshigaki var rosea., yeast-water contribute very good nitrogen source as stated above, but we need some other source of nitrogen in practical use of this fermentation. For this purpose "Hoshigaki" extract, soy bean extract and extract of rice bran were tested. As the result, in the case of "Hoshigaki" extract, the most favourable act was found when it gave 0.0077 g. of nitrogen to 100 c.c. of the medium and obtained as such 0.0196 g. in the case of soy bean extract, while the extract of rice bran contributed the most inferior source of nitrogen as we have gained lower yield of acid even in addition of 0.1318 g. of nitrogen to 100 c.c.

The preparation of extracts.

(1) "Hoshigaki" extract. To "Hoshigaki" ten times of water in quantity was added and warming in water bath several hours, adding water frequantly to recover the loss by evaporation.

The extract show 4° of Balling, containing 3 g. of glucose and 0.0231 g. of nitrogen in 100 c.c. To this extract 8 g. glucose (Ozawa) was added to 100 c.c.

(2) Soy-bean extract. To soy-beans two times of water in quantity was added and warmed in water bath as extract (1). The extract (8°B) contained 0.1178 g. of nitrogen in 100 c.c. This was diluted in several ways to get many media with different contents of nitrogen, adding 13 g. glucose (Ozawa).

(3) Rice bran extract. It was prepared in quite same way as soy-bean extract.

It gave 2° of Balling, and contained 0.0659 g. of nitrogen in 100 c.c. of it. A part of the extracts was evaporated to prepare more higher contents of nitrogen. Glucose was added as in the case of soy-bean extract.

The cultures were incubated at 26~28°C during 18 days. The results are recorded in the table below :—

(I) "Hoshigaki" extract (100 c.c.)

No.	Glcose. g.	Nitrogen. g.	Volatile acid c.c. of n/10 NaOH.	Non. Vol. acid c.c. of n/10 NaOH.	Glucon. calcu. g.	Ca-gluc. Calc. g.	Ca-gluc. found. g.	$\frac{\text{Glucon. a.}}{\text{Tot. Sugar.}} \times 100$
I.	7.65	0.0694	4.0	210.0	4.11	4.68	5.0	53.7
IV.	8.35	0.0115	4.7	405.3	7.94	9.05	9.4	95.0
V.	**8.22**	**0.0077**	**4.0**	**430 0**	**8.42**	**9.59**	**9.6**	**102 4**

(II) Soy-bean extract. (100 c.c.)

I.	8.65	0.1178	13.2	397.3	7.79	8.88	9.0	90.0
III.	8.65	0.0392	8.7	409.9	7.93	9.04	9.1	91.6
V.	**8.65**	**0.0196**	**4.8**	**456.2**	**8.94**	**10.10**	**10.0**	**103.4**

(III) Rice bran extract. (100 c.c.)

I.	**8.65**	**0.1318**	**4.5**	**388.5**	**7.41**	**8.44**	**8.7**	**85 6**
II.	8.65	0.0659	3.1	357.9	7.01	7.99	8.2	81.0
V.	8.65	0.0109	3.0	220.0	4.31	4.91	5.1	49.8

N. B. If we increase the contents of nitrogen in rice bran extract, we can expect better yield than tabulated.

The quantity of glucose in the table is the result of quantitative determination prior to the experiment. The methods of determination of acids are quite same as mentioned above.

Literature.

(1) L. Boutroux: C. r. ac. Sc, **86** 605, (1878).
〃 〃 〃 〃 〃 **91** 236, (1880).
Ann. Pasteur: Watt's Dictionary of Chemistry. **2** 613, (1888).
(2) A. J. Brown: Jour. Chem. Soc., **49**, 172, u. 432, (1886).
(3) Seifert: Centralbl. f. Bakt., (1897).
(4) W. Henneberg: Zeit. f. Essigind. u. Centralb. f. Bakt., 2 Abt. **4**.14 (1898).
(5) A. Zeidler: Zent. f. Bakt., 2 Abt., **2** 729, (1896).
(6) D. P. Hoyer: Delft (1898); durch. Die deut. Essigind., **3** 1, (1899).
(7) Beijerink: Centralbl. f. Bact., **29** 169, (1911).
(8) K. Miyaji: Agric. Soc. of Japan., **225** 233~309. (1922); **238** 523~682; **256** 160, (1924).
(9) K. Yagi and Hashitani: Kogio Kwagakukwaishi, (1923).
(10) S. Hermann: Bioch. Zeit, **192** 1~3. Heft. 176~199 (1928); u. **205** 4~6 Heft. 297, (1929).
(11) R. Falk and S. R. Kapur. **57** 920 (1924).
(12) K. Bernhauer: Bioch. Zeit., **172** 313, 324, (1926).
(13) C. Wehmer: Bioch. Zeit., **117** 4~6 Heft. 418~432 (1928).
(14) K. Sakaguchi: Nippon Gakujutsu Kyōkwai. (1929).
(15) O. E. May: Thom and M. B. Church. Jour. Biol. Chem., 417 (1927).
(16) O. E. May and H. I. Herrick: Jour. Biol. Chem., 77, 185~195 (1928).
(17) T. Takahashi and K. Sakaguchi: Agr. Chem. Soc. Japan, Vol. III. No. 31, 540.
(18) S. Hermann: 1. c.
(19) T. Takahashi and K. Sakaguchi: Agric. Chem. Soc. Japan., Vol. I. No. 14.
(20) Dō: Agric. Chem. Soc. Japan, Vol. III. No. 31, 540.
(21) Herzfeld: A., 220, 345, 347, 350.
(22) Bertrand: A. Ch. (8) 3, 275 (Beilstein: VI. Aufl. III, S. 544.).
(23) Fischer: B., **23** 2611.
(24) Führmann. u. Hermann: Bioch. Zeit., 192, 1~3 Heft. 176~199 (1928).
(25) Beijerinck: Centralbl. f. Bact., 2 Abt. Bd. 4, 214. (1898).
(26) L. Pasteur: Etudes sur le vinaigre., 18, (1864).
(27) W. Henneberg: Gäringsbacteriologie, 2 Teil. 228, (1928).
(28) Janke-Zikes: Arbeit Methoden der Mikrobiologie 115. (1928).
(29) 〃 〃 〃 〃 〃 〃 〃 〃
(30) Hermann: Bioch. Zeit., 192, 1~3 Heft. 176~199 (1928).

ON GLUCONIC FERMENTATION. PART II.

ON THE FORMATION OF OXY-GNUCONIC ACID. (5-KETO-GLUCONIC ACID).

By

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(Received October 15 th., 1930)

In 1886 Boutrox(1) found a new organic acid from fermentation product of his Micrococcus oblongus, which was cultured in the yeast water contaning glucose or gluconic acid associated with Ca-carbonate in either case. The acid, having a ketonic radical and laevo-rotatory power with the formula of $C_6H_{10}O_7$ or assumed constitutional formula of $CH_2OH{\cdot}CO{\cdot}OH{\cdot}CH{\cdot}CH{\cdot}OH{\cdot}OH{\cdot}CH{\cdot}COOH$ was named oxy-gluconic acid (α–β–γ–ω tetroxy–δ–oxo–pentan–α –carbonic acid).

Bertrand's memoir(2) about this acid, as the product of his sorbose bacteria from gluconic acid at 18～25°C, was about a decade later from pioneer's work.

Ruff's(3) chemical preparation of this acid from Ca-gluconate by oxidising with bromine or hydrogenperoxide in presence of basic ferric acetate was unsatisfactory, as its yield was limited near 1%, giving rise many byproducts such as arabinose, glycollic acid and formic acid. On the other hand, Killiani4) prepared the same in the most purest state from glucose by oxidising it with nitric acid. The yield was better than former as such 15～16%.

Quite recently, S. Hermann(5) in the study of his Bact. gluconicum found about 70% of 5-keto-gluconic acid from glucose, gluconate, free gluconic acid and fructose in the culture of three months old. In the same year K. Bernhauer aud K. Schön(6) took advantage to find out the same acid from glucose in the culture of Bact. xylinum in presence of Ca-carbonate.

In the former publication(7) of authors on gluconic fermeatation, thers was mentioned four new acetic bacteria and one of them viz. Bact. Hoshigaki var rosea was described in details. The other three varieties, including Bact. industrium var Hoshigaki, happened to give rise oxygluconic acid from glucose in presence of Ca-carbonate.

Experimental.

To 100 c.c. of yeast water*, 10g. of glucose (Merck) and 2.5g. of Ca-carbonate was added and the three varieties of authors' bacteria were seeded, provided the necessary pasteurisation of the the medium.

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* To 50 g of pressed beer yeast, 1000 c.c. of tap water was added and taken in water bath heated to boiling during two hours. 10 c.c. of it wanted 0.6 c.c. of n/10 of NaOH.

The cultures were incubated at 26~28°C and after 20 days, the carbonate added in the flask completely dissapeared, especially in the culture of Bact. industrium var Hoshigaki, owing to the formation of some acid. After that, standing these cultures at room temperature (18~20°C) for sevesal days, plentiful crystals developed covering the whole surface of the bottom of flask.

Isolation and identification of the acid.

The crystals of salt, with the shape of oblique plates or prisms, obtained from the culture of Bact. industrium var Hoshigaki, were washed several times with distilled water prior to the drying at 100°C. The acid under examination was free from nitrogen and its salt gave just calcium as the ash on ignition.

The properties shown below are given by the free acid thus : ——

(1) It reduces Fehling's solution gradually even at room temperature, but suddenly upon warming.

(2) It reduces ammoniacal silver nitrate when warmed.

(3) Sodium nitroprusside in the presence of ammonia, when warmed gives a dark yellow green colouration to the acid under examination.

(4) Sodium nitroprusside, warmed in presence of caustic potash with the acid under examination, gives a red colouration, which changes to green when acidified by acetic acid.

(5) The solution of neutral salt of the acid under examination, waring after the addition of ferric chloride, takes a red colour which changes to yellow by cooling,

(6) It gives Bial's orcin reaction(7) and Wheeler and Tollen's phloroglucine reaction(8) for pentose.

(7) It gives furfurol when digested with concentrated HCl. The furfurol after distillation is proved by well known colour reaction by anilin acetate or barbituric anilin acetate.

(8) Seliwanoff's(9) reaction which is specific one for ketose, is positive by this acid.

From these reactions given above, the conclusion may be drown most safely that the acid under examination should be one of the ketonic acid with six carbon atoms in the molecule. Further, its specific rotatory power is − 14.3° and it dose not form a crystalline lactone, although it has been evaporated at low temperature under reduced pressure.

It colours brown by simple warming, but in presence of alkali decomposes changing to a dark brown mass. It dissolves easily in water and alcohol, but almost insoluble in ether. These characteristics remind us Boutroux's oxygluconic acid and its identification follows from the description below.

(1) *Specific rotatory power of the acid.* Free acid rotated the polarized light 14.3° to the left, thus :——

$C=9.76$ $l=0.5$ dm. $t=18C$ $\alpha=-0.70°$

$$[\alpha]^{18°}=\frac{\alpha\times 100}{C\times l}=\frac{-0.7\times 100}{9.76\times 0.5}=-14.3°$$

After Ruff(10) $[\alpha]_D=-10.3°$ (2% Ca-salt with HCl)

$[\alpha]_D>-12.8$ (free acid)

After Boutrox(11) $[\alpha]_D=-14.5°$ $(C=2)$

(2) *Calcium and the water of crystallization of Ca-salt.* The Ca-salt was obtained from aqueous solution of potassium salt by the addition of quantitative necessary Ca-acetate. It appears as beautiful oblique prism or plate, which dried at 100°C give 8.26% of calcium shown below :——

Substance taken 0·6269 g. $CaCO_3=0.1259$ g. $Ca=0.0518$ g.

Calc. as $(C_6H_9O_7)_2$ $Ca+3H_2O$ Ca. 8.34%

Found. 〃 8.26%

Thus the water of crystallization of the salt must be three molecules and it was substanciated by drying the salt at the temperature of 100∼140°C.

The results are given below :——

Temp. C.	Substance.	Decrease by weight	Loss of water. %	Calc. as	Water of crystallization. %
	g.	g.			
100	0.5181	0	0		
100∼115	0.5107	0.0074	1.42	$(C_6H_9O_7)_2Ca.\ 1H_2O$	3.78
120∼130	0.4793	0.0388	7.49	$(C_6H_9O_7)_2Ca.\ 2H_2O$	7.56
130∼140	0.4596	0.0585	11.30	$(C_6H_9O_7)_2Ca.\ 3H_2O$	11.34

N. B. The substance at 120∼130°C gained somewhat yellow color and at 130∼140°C changed to brown, indicating the decomposition although in trace.

The calcium salt dehydrated contained 9.46% of calcium, identifying to that of Ruff's(12) and Boutroux's(13) Ca-salt of oxygluconic acid.

(3) The result of *elementary analysis of the Ca-salt* is described below.

Substance taken 0.2200 g.	$CO_2=0.2411$ g.	$H_2O=0.1014$ g.
	C.	H.
Calc. as $(C_6H_9O_7)_2Ca+3H_2O$	29.98%	5.03%
Found.	29.88%	5.12%

(4) *Strontium salt.* Strontium salt was prepared by the addition of strontium acetate to the aqueous solution of potassium salt.

It crystallized in oblique prism and after recystallizing from hot water, was dried at 100°C prior to the determination of strontium. The result is shown below :——

Substance taken	0.3190 g.
$SrSO_4=0.1191$ g.	$Sr=0.0568$ g.
Sr calc. as $(C_6H_9O_7)_2SrH_2O$	17.81%
Found.	17.80%

Thus, the water of crystallization of this salt seems to be just one molecule, showing discrepancies to the result of Boutroux(14), whose Sr-oxygluconate contained 3 or $1\frac{1}{2}$ molecules of water of crystallization.

(5) *Lead salt.* This salt was obtained from its potassium salt by additein of lead acetate. The salt dried at 100°C, contained no water of crystallization as shown below :——

Substance taken	0.3380 g.
$PbSO_4=0.1737$ g.	$Pb=0.1186$ g.
Found.	Pb 35.09%
Calc. as $(C_6H_9O_7)_2Pb$	Pb 34.92%

Lead salt of Boutroux's(15) oxygluconic acid contained 1 or 2 molecules of water of crystallization.

BIBLIOGRAPHY.

(1) Boutroux: C. r. **102** 924, (1886); **104** 369, 114, 185 et An. d. Ch., (6) **21** 565, (1890).
(2) Bertrand: A. Ch. **3**, 281.
(3) O. Ruff: Ber. **32** 2269.
(4) Killiani: Ber. **55** 2820, (1922).
(5) Hermann: Bio. Zeit., Bd. 205, 4~6 Heft. S. 297 (1929).
(6) Takahashi et Asai: Journ. Soc. of Brew. Japan. No. 1, No. 2, No. 3, (1930).
(7) M. Bial: Med. Woch. **28** 253 (1902).
(8) H. J. Wheeler u. B. Tollens: Lieb. Ann. **254** 329 (1889).
(9) Seliwanoff: Ber. **20** 181 (1887).
(10), (12) O. Ruff: Ber., **32** 2269.
(11), (13), (14), (15): Boutroux: Ann. Ch. (6) **21** 572.
(14), (15) Lipmann: Die Chemie der Zuckeraten I, 324 (1904).

ON THE NATURAL PIGMENTS OF RAW SILK FIBRE OF THE DOMESTIC COCOON. (PART II)

XANTHOPHYLL OF MULBERRY LEAVES AS A SOURCE OF YELLOW COCOON XANTHOPHYLL.

By

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(Received November 4 th., 1930).

In previous papers I have reported the occurrence of xanthophyll in the domestic yellow cocoon and the coincidence of this pigment with that of plant origin, formerly studied by Willstätter and others. I have also discussed there that xanthophyll of the yellow cocoon should be probable to be derived biologically from that of mulberry leaves upon which the silk worms feed.

In this research I have isolated xanthophyll from mulberry leaves in a good yield and identified it as wholly coincides with that of yellow cocoons.

Experiments.

1 kg. of fresh mulberry leaves (plucked off on 6 th, Sept.) was finely chopped and soaked in 5 L. of 90% ethyl alcohol in a large beaker and digested under 15 lbs. pressure for half an hour in an autoclave, about 60% alcohol was taken as a boiling medium, instead of water. After filtration on a Büchner funnel, the macerated leaves were further treated with boiling alcohol and filtered as before and then washed with warm alcohol until the macerated leaves became almost colorless. All the alcoholic extract of the chloroplast pigments thus obtained was concentrated under reduced pressure, passing carbon dioxide to avoid the oxidation of the pigments. To the condensed pigments suspension, ether added and chlorophyll and carotinoids were transferred to the ethereal layer. After condensation and complate saponification with methyl alcoholic potash, the ether solution was washed with water to remove potassium chlorophyllines completely. Ether solution was then dehydrated by anhydrous sodium sulphate and evaporated to dryness, upon which 600 c.c. of petroleum ether (b.p. 40∼60°) was added and stirred well. Thus crude xanthophyll was precipitated, dissolving carotin and waxy substances in petroleum ether fraction.

Boiling this crude xanthophyll with low petroleum ether, pure crystalline xanthopoyll was obtained by recrystallisation from boiling methyl alcohol.

The yield was 0.165 g. viz. 0.044% of the anhydrous sample which showed about 73% of the theoretical value. (Colorimetric determination.)

In another experiment I have obtained 0.142 g. of crystalline xanthophyll from 500 g. of air dried mulberry leaves, (10.25 % moisture content) which showed in yield 73% of that, obtained from fresh leaves. Thus, xanthophyll in the green fresh leaves was considered to be oxidised during the air drying. Therefore, the preparation must be recommended to start from fresh leaves.

The crystalline xanthophyll thus isolated showed very similar coloration and crystal form with that, isolated from yellow cocoon and proved its melting point to be 175° (uncorr.) which revealed no change by mixed probe with cocoon xanthoyhyll.

Elementary analysis: —	C% 84.24	H% 10.08
Molecular weight: —	537.2	

The absorption bands of the alcoholic solution of this xanthophyll coincides wholly with that of cocoon xanthophyll.

Judging from the results obtained, the origin of xanthophyll in the domestic yellow cocoon can be asked in the mulberry leaves upon which the silk worms feed.

The new extraction method of chloroplast pigment from fresh green leaves, adopted in the above experiment, can be considered to be recommended widely.

STUDIEN ÜBER DIE GÄRUNGSPRODUKTE DER SCHIMMELPILZE.

VIII. MITTEIL. ASPERGILLUS GLAUCUS. (III).

von

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(Eingegangen am 18 November 1930).

In früheren Mitteilung (Bull. agrikul. chem. Soc. Japan, **5**, 10, 1929) wurde ein Verfahren beschrieben, dass bei der Kurtur von Asp. glaucus die

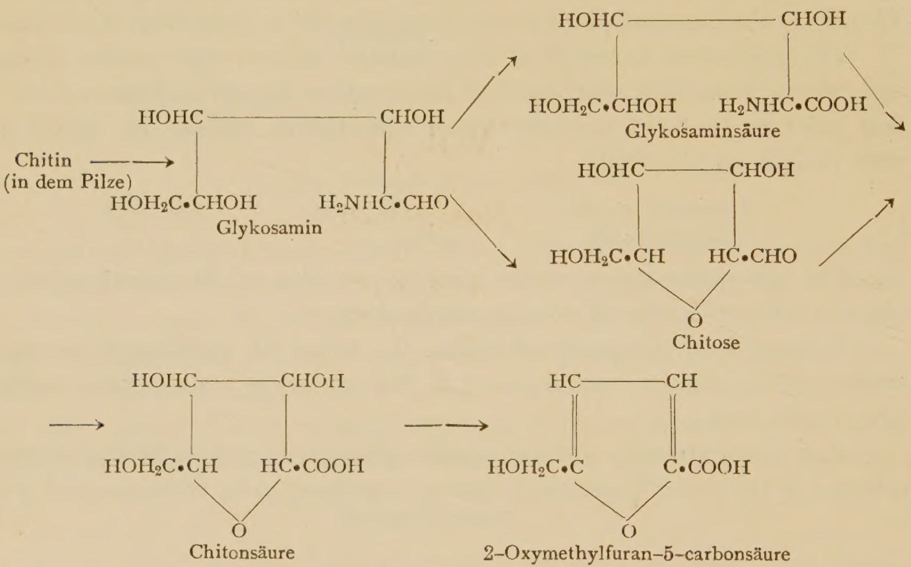
2–Oxymethylfuran-5-carbonsäure in kleinen Menge gebildet ist und identifiziert wird.

In diesem Bericht wird die Mechanik von der Produktione der 2–Oxymethylfuran–5–carbonsäure erklärt. Mann kann vorschlagen folgende drei Mechaniken.

(1) Glykose→Glykonsäure→(Chitonsäure→)2–Oxymethylfuran–5–carbonsäure.

(2) Glykose——→Oxymethylfufurol——→2–Oxymethylfuran–5–carbonsäure.

(3)



Bei allen Zuchtungen von Asp. glaucus in der Glykonsäure oder Oxymethyl–furfurol erhaltende Nährlösungen als Kohlenstoffquelle wird die 2–Oxymethylfuran–5–carbonsäure nicht gewonnen. Aber bei der Kurturen von Asp. glaucus in der Nährlösungen, welche Glykosamin, Glykosaminsäure, Chitose oder Chitonsäure erhalten sind, wird die 2–Oxymethylfuran–5–carbonsäure immer gewonnen und identifiziert. Ueberdies wird die Chitonsäure aus Glykosamin und Chitose mit der 2–Oxymethylfuran–5–carbonsäure mittels des Pilzes gebildet. Es zeigt sich deutlich, dass die erste und zweite Mechaniken irrig sind und nur der dritte rechtig ist.

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